CHS-114, an Anti-CCR8 Antibody That Selectively Depletes Intratumoral Tregs and Induces Antitumor Immune Responses

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Poster 202-B

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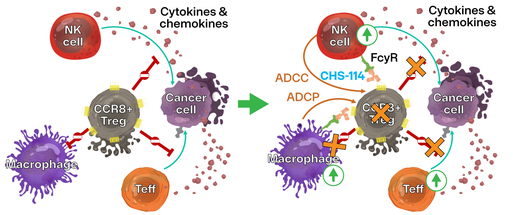
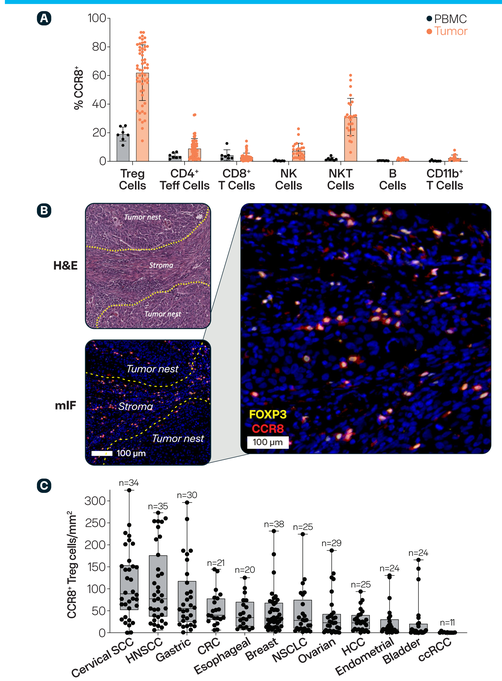
Introduction

- FOXP3⁺ regulatory T cells (Tregs) play a crucial role in modulating immune responses across several tissues, including the tumor microenvironment (TME).[1,2]
- Broad depletion of Tregs may elicit deleterious autoimmunity; however, selective depletion of Tregs within the TME may enhance antitumor immune responses.
- CCR8 is a 7-transmembrane G protein-couple chemokine receptor (GPCR) that is predominantly upregulated on tumor-resident Tregs. Preferential depletion of Tregs in the TME may be achieved through targeting of CCR8.[3,4]
- CHS-114 is a highly selective, effector-enhanced, human anti-CCR8 afucosylated mAb that lacks off-target binding and exhibits no cross-reactivity to CCR8 expressed in other species.
- CHS-114 is currently in Phase 1 clinical studies for advanced cancer patients (NCT05635643).



Intratumoral Tregs Highly Express CCR8



(A) CCR8⁺ cell frequency in immune cell subsets as determined by flow cytometry from healthy PBMC (n = 14) and dissociated primary tumor tissues (n = 55). Error = SD. **(B)** Multiplex immunofluorescence (mIF) evaluating FOXP3 and CCR8 shows abundant CCR8⁺ Tregs in the TME of an HNSCC tumor sample. Representative images at 10x (H&E and low-power mIF) and 20x magnification (high-power mIF). **(C)** The density of CCR8⁺ Tregs in 12 types of solid tumors was determined by mIF staining.

Abbreviations: 2L = second line; ADCC = antibody-dependent cellular cytotoxicity; ADCP = antibody-dependent cellular phagocytosis; BOIN = Bayesian optimal interval; CCR8 = chemokine receptor 8; ccRCC = clear cell renal cell carcinoma; CFSE = carboxyfluorescein succinimidyl ester; CR = complete response; CRC = colorectal cancer; CxDy = Cycle x Day y; DC = dendritic cell; DL = dose level; DLT = dose-limiting toxicities; DTC = dissociated tumor cells; FC = flow cytometry; gMFI = global mean fluorescence intensity; GPCR = G protein-coupled receptor; H&E = hematoxylin and eosin; HCC = hepatocellular carcinoma; HNSCC = head and neck squamous cell carcinoma; IFN = interferon; IL2 = interleukin-2; IO = immuno-oncology; IQR = interquartile range; mAb = monoclonal antibody; MFI = mean fluorescence intensity; mIF = multiplex immunofluorescence; MSD = meso scale discovery; NK = natural killer; NSCLC = non-small cell lung cancer; PBMC = peripheral blood mononuclear cells; PD-1 = programmed cell death protein 1; RECIST = Response Evaluation Criteria in Solid Tumours; SCC = squamous cell carcinoma; scRNAseq = single cell RNA sequencing; SD = standard deviation; SEM = standard error of the mean; Tconv = conventional T cell; Teff = effector T cell; TGI = tumor growth inhibition; TIL = tumor-infiltrating lymphocytes; TME = tumor microenvironment; TNF = tumor necrosis factor; Treg = regulatory T cell.

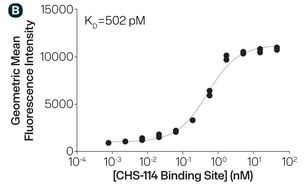
References: **1)** Plitas et al, *Ann Rev Can Biol* 2020. **2)** Sakaguchi et al, *Ann Rev Imm* 2020. **3)** Plitas et al, *Immunity* 2016. **4)** Wang et al, *Nat Imm* 2019.

Statistics: * = p < 0.05; ** = p < 0.01; *** = p < 0.001; **** = p < 0.0001; when not shown, comparisons are not statistically significant.

CHS-114, a Human Afucosylated IgG1 Antibody, Selectively Binds Human CCR8 With High Affinity and Induces ADCC and ADCP

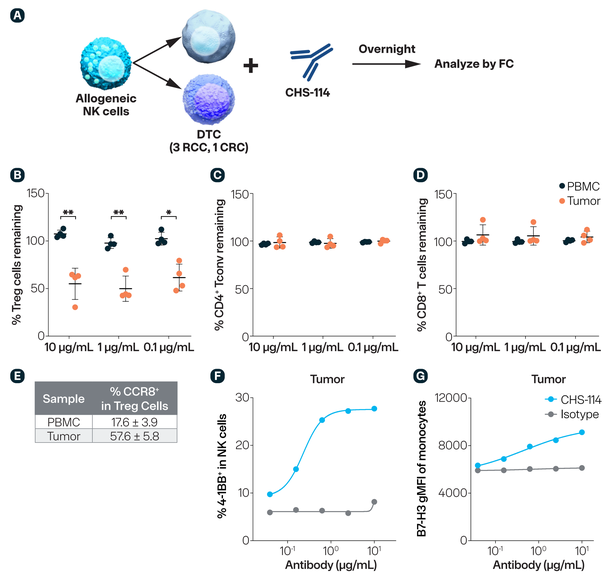
A

Selected Antibodies	Non-CCR8 Targets Identified
CHS-114	0
mAb2	1
mAb3	8
mAb4	15
mAb5	20
Comparator Antibodies	**Non-CCR8, "Off Target" Binding**
Comparator 1	ANGPTL7
Comparator 2	J chain
Comparator 3	SEMA4B



(A) Antibody specificity screen evaluating binding to 5,528 extracellular protein targets by cell microarray demonstrates that CHS-114 has no off-target binding compared to other CCR8 antibody candidates (includes evaluation of 3 clinical-stage CCR8 mAbs derived from patent sequences). **(B)** To assess binding affinity of CHS-114 to human CCR8, engineered human CCR8 expressing 293T cells were incubated with CHS-114 and measured by flow cytometry (FC). **(C)** To assess CHS-114-mediated ADCC, labeled Raji⁻ hCCR8 cells were co-cultured with NK cells and treated with CHS-114 or isotype control for 4 hours and then analyzed by FC. % NK killing represents the frequency of viable dye⁺CFSE⁺Raji⁻ hCCR8 cells among total CFSE⁺Raji⁻ hCCR8 cells. **(D)** To assess CHS-114-mediated ADCP, CFSE-labeled Raji⁻ CCR8 cells were co-cultured with monocyte-derived macrophages and were treated with CHS-114 or isotype control for 3 hours and then analyzed by FC. % phagocytosis represents the frequency of CD14⁺CFSE⁺ macrophages among total CD14⁺ macrophages. Error = SD.

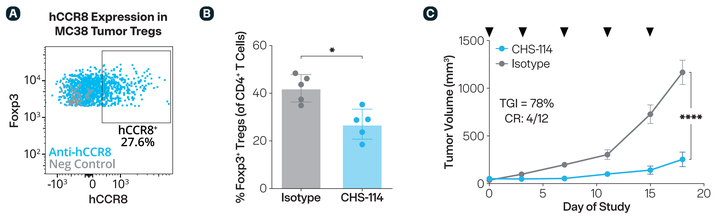
CHS-114 Depletes Intratumoral Tregs and Activates NK Cells and Monocytes



Sample	% CCR8⁺ in Treg Cells
PBMC	17.6 ± 3.9
Tumor	57.6 ± 5.8

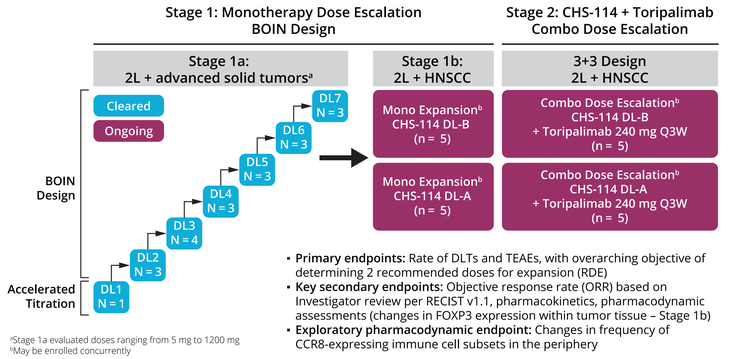
(A) Schematic representation of the experimental system; dissociated tumor cells (DTC) from human tumors. **(B-E)** Allogeneic human NK cells were activated with IL-2 and added to either healthy human PBMC or DTC (3 RCC, 1 CRC, n = 4). Co-cultures were treated with CHS-114 or isotype control overnight and analyzed by flow cytometry. % remaining Tregs **(B)**, CD4⁺ Tconv cells **(C)**, and CD8⁺ T cells **(D)** were calculated by dividing the frequency of CHS-114 treated cells by the frequency of isotype-treated cells. **(E)** Frequencies of CCR8⁺ Tregs in PBMC and tumor from the total Treg cell population (untreated). **(F)** NK and **(G)** monocyte activation in DTC (RCC) that were treated with CHS-114 or isotype control (representative results are shown). Statistical analysis was performed using unpaired t test. Error = SD.

Treatment With CHS-114 Results in Significant Antitumor Activity in Human CCR8 Knock-In Mice



MC38 cells were injected into the flank of CCR8 knock-in mice subcutaneously. Mice were dosed with either CHS-114 (10 mg/kg) or isotype control on Day 6 post implantation. **(A)** Representative flow cytometry readout of hCCR8 expression on FOXP3⁺ tumor Tregs on Day 10 after implantation. **(B)** Frequency of tumor FOXP3⁺ Tregs in dissociated MC38 tumor on Day 4 after treatment initiation (n = 5). **(C)** Tumor size assessment following treatment with CHS-114 or isotype control (n = 12/group). Error = SEM. TGI = tumor growth inhibition; CR = complete response (no evidence of palpable tumor). **(D)** Change in relative frequency of immune cells after treatment with CHS-114 compared to isotype control as identified by single cell RNA sequencing (scRNAseq) analysis of MC38 TILs. CD45⁺ TILs were isolated on Day 6 after treatment and scRNAseq analysis was performed using 10x Genomics (n = 2-3 replicates, 5 pooled tumors per replicate). Statistical analysis was performed using unpaired t test.

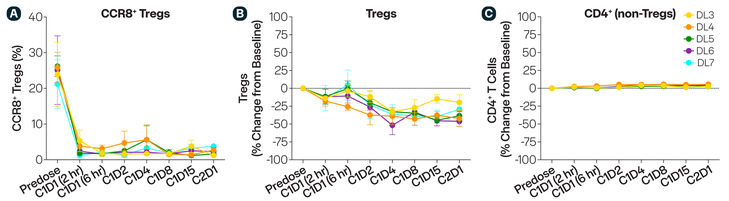
CHS-114-101 Phase 1 Clinical Trial Schema



[a]Stage 1a evaluated doses ranging from 5 mg to 1200 mg
[b]May be enrolled concurrently

- **Primary endpoints:** Rate of DLTs and TEAEs, with overarching objective of determining 2 recommended doses for expansion (RDE)
- **Key secondary endpoints:** Objective response rate (ORR) based on Investigator review per RECIST v1.1, pharmacokinetics, pharmacodynamic assessments (changes in FOXP3 expression within tumor tissue – Stage 1b)
- **Exploratory pharmacodynamic endpoint:** Changes in frequency of CCR8-expressing immune cell subsets in the periphery

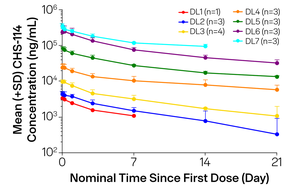
Sustained and Selective Peripheral CCR8⁺ Treg Depletion Observed at ≥ DL3



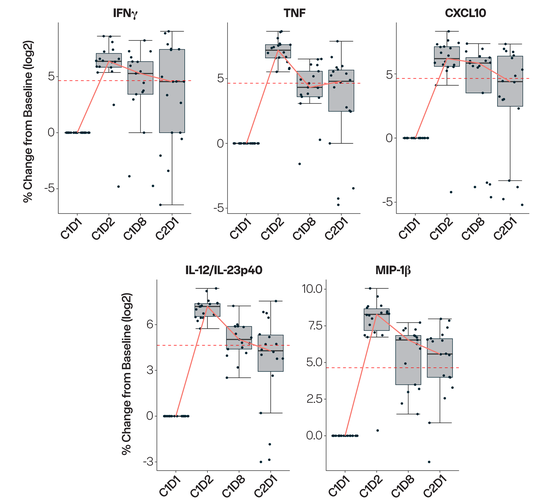
CCR8⁺ Tregs in the PBMC were measured from whole blood using flow cytometry before CHS-114 treatment (predose) and at indicated timepoints throughout CHS-114 Cycle 1 for Dose Levels (DL)3–DL7. **(A)** CCR8⁺ Treg depletion (> 85%) in the periphery was stable through Cycle 1 for all dose levels tested. Additionally, depletion was observed at DL3 (and higher doses), which was lower than predicted dose from *in vitro* modeling. **(B)** CHS-114 treatment led to a decrease in subset of Tregs, while preserving broader Treg and non-Treg CD4⁺ T cell populations **(C)**, confirming the specificity of CHS-114 for CCR8⁺ Tregs. Tregs were defined as $CD127^{low}$ $CD25^{high}$ cells within the $CD3^{+}$ $CD4^{+}$ T cell population. Data representative of the average per dose level (n = 2-3 participant samples per timepoint). Error = SEM.

CHS-114 Concentration After First Administration

CHS-114 PK exposure increases with dose. Dose proportionality is observed at all dose levels. PK elimination is linear over all dosing levels with a half-life of approximately 10 days (range: 9-17 days).



CHS-114 Administration Promotes Immune Activation in Heavily Pretreated Cancer Patients



CHS-114 treatment of cancer patients induces proinflammatory cytokines. Serum cytokines were measured by MSD assay at indicated time points following the first administration of CHS-114 for patients enrolled in SRF114-101 monotherapy dose escalation (C1D1, before drug administration/predose). A sustained increase in proinflammatory cytokines (IFNγ, TNF, CXCL10, IL-12/IL-23p40, and MIP1β) compared to pretreatment levels across the dosing levels of CHS-114 was observed and is consistent with inducing an immune response. Data representative of 18 patients (at baseline). Error bars represent the interquartile range (IQR). Dashed red horizontal line: +25% change from baseline. All assays were significant at C2D1 relative to C1D1 with Wilcox test p-values < 0.05. Median values are depicted as a solid red line.

Conclusions

- CCR8 is a GPCR that shows preferential expression on tumor resident Tregs and has promise as a drug target for selectively targeting immune suppression in the TME.
- CHS-114 is a novel afucosylated human IgG1 mAb that selectively and potently targets human CCR8 with no off-target binding and has the potential to overcome Treg immune suppression in the TME by depleting Treg cells and potentially increasing the T cell:Treg ratio in the tumor, enabling antitumor immunity.
- In dissociated tumor cells, CHS-114 activates NK cells and specifically induces cytotoxicity against tumor-infiltrating Tregs.
- The mechanism of action is demonstrated in a humanized CCR8 mouse tumor model: CHS-114 treatment results in significant antitumor activity and depletes human CCR8⁺ Tregs, resulting in the expansion of CD8⁺ T cells and activation of myeloid cells.
- CHS-114 has demonstrated an acceptable safety profile in heavily pretreated patients with advanced solid tumors, with no DLTs reported to date and TEAEs generally low grade.
- CHS-114 human PK parameters increase with dose, is approximately dose proportional, and the elimination appears linear with a half-life of about 10 days (range 9-17 days).
- Depletion of peripheral CCR8⁺ Tregs was observed in cancer patients and depletion was maintained over the dosing interval, establishing proof of mechanism.
- CHS-114 induces proinflammatory cytokines over the dosing interval, implying immune activation that is associated with depletion of CCR8⁺ Tregs.
- Preliminary results and acceptable safety profile support further evaluation of CHS-114 in combination treatment with the anti-PD-1 antibody, toripalimab, and other IO agents.